

Colorectal carcinogenesis: MSI-H versus MSI-L

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Abstract. Microsatellite instability (MSI) is a well-recognized phenomenon that is classically a feature of tumors in the hereditary non-polyposis colorectal syndrome. Ten to 15% of sporadic colorectal cancers, however, will have MSI. Microsatellite unstable tumors can be divided into two distinct MSI phenotypes: MSI-high (MSI-H) and MSI-low (MSI-L). MSI sporadic colorectal cancers with a high level of MSI (MSI-H) form a well defined group with distinct clinicopathologic features characterized by an overall better long-term prognosis. These sporadic MSI-H colorectal tumors most often arise from the epigenetic silencing of the mismatch repair gene MLH1. In contrast, MSI-L colorectal tumors have not been shown to differ in their clinicopathologic features or in most molecular features from microsatellite stable (MSS) tumors. Unlike MSI-H tumors, MSI-L tumors appear to arise through the chromosomal instability carcinogenesis pathway, similar to MSS tumors. Some groups have reported more frequent mutations in K-ras and in the methylation of methylguanine transferase in MSI-L tumors, but others have questioned these findings. Therefore, although the use of the MSI-L category is widespread, there continues to be some debate as to whether a discrete MSI-L group truly exists. Rather, it has been suggested that MSI-L tumors differ quantitatively from MSS tumors but do not differ qualitatively. Future studies will need to evaluate the specific mutations in non-MSI-H tumors in an attempt to sub-classify MSI-L tumors with regard to MSS tumors so that subtle differences between these two sub-groups can be identified.

1. Introduction

Colorectal cancer is both the third most common type of cancer, as well as the third leading cause of cancer-related deaths in North America [1]. In 2003, an estimated 145,000 new cases of colorectal cancer will be diagnosed with an associated 58,000 colorectal deaths [1]. The malignant transformation associated with colorectal carcinogenesis involves the stepwise accumulation of multiple genetic alterations that allow the growth of neoplastic cells [2–4]. Currently, adenocarcinomas of the colon and rectum are believed to arise from two distinct molecular genetic pathways: one involving chromosomal instability (CIN) and the other involving microsatellite instability (MSI) [5–8]. CIN results from a series of genetic changes that involve

the activation of proto-oncogenes such as K-ras, and inactivation of tumor-suppressor genes, such as APC, TP53, DCC, SMAD2, SMAD4 [2,9–12]. CIN tumors are characterized by a high frequency of allelic imbalance (most commonly involving chromosomal arms 5q, 8p, 17p, and 18q), chromosomal amplifications, and translocations [2]. Seventy-five to 80% of sporadic cases of colorectal carcinoma arise through the CIN pathway [9,10].

The second pathway of colorectal carcinogenesis is the MSI pathway. In the MSI mutational pathway, colorectal cancers display increased rates of intragenic mutations of short, tandemly repeated DNA sequences known as microsatellites [13–15]. The phenomenon of MSI results from inactivation, mutational and/or epigenetic silencing of mismatch repair (MMR) genes. The primary function of the postreplicative MMR is to eliminate base-base mismatches and insertion/deletion loops that arise as a consequence of DNA polymerase slippage during DNA synthesis [10, 16,17]. Mutation rates in tumor cells with MMR deficiency are 100- to 1,000-fold more common as com-

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pared to normal cells [18,19]. The vast majority of hereditary non-polyposis colorectal cancer (HNPCC) patients will have microsatellite high (MSI-H) tumors. HNPCC is an autosomal-dominant condition that accounts for approximately 2–3% of all colorectal carcinomas. In most cases, the genetic basis for instability in MSI tumors is an inherited germline alteration in any one of the five human MMR genes: MSH2, MLH1, MSH6, PMS2, and PMS1 [20–23]. More specifically, germline mutations in MSH2 and MLH1 are responsible for most HNPCC families, while MSH6 is less common and PMS2 and PMS1 are rare [20,22].

MSI-H is also seen in 10–15% of sporadic non-HNPCC colorectal cancers. In patients with MSI-H sporadic colorectal cancer, the basis for instability is usually an acquired hypermethylation of the MLH1 promoter with subsequent transcriptional silencing [24–28]. MSI-H sporadic colorectal cancers do not exhibit gross cytogenetic abnormalities, infrequently display allelic losses at tumor suppressor loci, and are usually not aneuploid [13,29–31]. Instead, MSI-H sporadic colorectal tumors accumulate mutations at microsatellite sequences in coding regions of specific genes that are implicated in tumor progression, such as *TGF β RII*, *IGFIIR*, *MSH3*, *MSH6*, and *BAX* [32–35].

2. Microsatellite Instability: High versus low

Criteria and guidelines used to define MSI have evolved and, to some degree, remain somewhat elusive. MSI was initially described in independent publications from three groups in 1993, each who used unique descriptors for their findings [13,15,36]. Since that time, there has been a concerted effort to develop international criteria for the determination of MSI in colorectal cancer [37]. Despite this, the number, type, and identity of the microsatellites which should be used for MSI assessment remain unclear and the criteria for scoring MSI continue to vary among studies [38]. There is, however, a consensus that some markers, including mononucleotide repeats such as BAT25, BAT26, and BAT40 and the dinucleotide repeats D5S346, D2S123, and D17S250, are particularly useful for the identification of MSI tumors [39–42].

More recently, several studies have shown that colorectal cancers demonstrating the MSI phenomenon can be divided into two distinct MSI tumor phenotypes: MSI-high (MSI-H) and MSI-low (MSI-L). This division depends on the proportion of markers showing

MSI and on the type of microsatellite marker that is utilized. In the MSI-H group, the majority of tested markers exhibit instability, whereas in the MSI-L group only a minority of markers show evidence of MSI. The distinction between these two groups is highly dependent on both the type and the number of microsatellites analyzed. For example, dinucleotide markers demonstrate both MSI-H and MSI-L, whereas mononucleotide markers such as BAT26 and BAT40 are relatively specific for MSI-H cancers [42,43]. Based on this, some groups have distinguished between the type of tandem repeat as a reliable means of defining MSI-L, or have used specific markers, such as the complex repeat MYCL, which is believed to be more specific for MSI-L tumors [42,44,45]. With regard to the number of markers demonstrating instability, most groups have defined MSI-H as those tumors with more than about 30–40% of markers unstable and MSI-L as a level of instability lower than this threshold [37,38,42,46].

Compared to MSI-L or microsatellite stable (MSS) tumors, MSI-H tumors have a distinct clinicopathologic phenotype [47–49]. MSI-H tumors tend to be poorly differentiated, right-sided, mucinous, have an expansile growth pattern, histologic heterogeneity, and increased tumor infiltrating lymphocytes with a prominent inflammatory reaction [5,50–53]. In addition, the carcinogenic pathway underlying MSI-H tumors (MMR repair defect) is believed to be distinct from the CIN pathway underlying non-MSI-H cancer. Based on these phenotypic as well as genetic differences, the distinction between MSI-H and non-MSI-H tumors has come to be universally accepted. In contrast, the MSI-L phenotype is not associated with distinct clinicopathological features or altered MLH1 or MSH2 expression and the biological basis of the MSI-L phenotype remains uncertain [28,37,42,44,54]. Given this, considerable controversy and debate remains around the validity and clinical utility of separating MSI-L tumors from MSS tumors.

3. MSI-L: A distinct sub-group of sporadic colorectal carcinoma

Some authors have argued that although MSI-L cancers may overlap more with MSS than MSI-H tumors, MSI-L still represents a distinct intermediate subset of colorectal cancers [43,55]. Although the MSI-L subset may not show distinct clinical, morphological, or molecular differences from the remaining MSS cancers (at least not to the degree seen between MSI-H and

non-MSI-H tumors), there are certain molecular features that are distributed in a nonrandom basis within the non-MSI-H subset of colorectal cancers [56]. In order to establish MSI-L as a distinct subset of non-MSI-H cancers, these molecular features would need to be identified in a group of tumors (i.e. MSI-L tumors) at a rate more than would be expected by chance. Jass and others have argued that when a panel of relatively sensitive markers is used, approximately 8% of sporadic colorectal cancers can indeed be designated as MSI-L [55,56]. Furthermore, some markers (D2S123, MYCL) are mutated at a much higher rate outside the MSI-H subset [57]. It is also evident that some colorectal cancers show more extensive MSI-L than would be expected by chance while others are “superstable” [58]. Therefore, nonrandomly distributed features among non-MSI-H cancers – including chromosomal alterations, DNA methylation, and the K-ras mutation – are hard to simply explain away by claiming that they are due to “more cell divisions” [55,59–62].

Kambara et al. examined the molecular events in early colorectal cancer with regard to MSI [60]. These investigators noted a significantly higher frequency of the low-level MSI (MSI-L) phenotype in early colorectal cancers (51.0%) compared with advanced colorectal cancers (25.9%; $p = 0.0001$) [60]. In early colorectal cancers, tumors with MSI-L phenotype were significantly different from MSS tumors with respect to loss of heterozygosity (LOH) at 1p32 (48.6% versus 18.9%) and 8p12-22 (71.1% versus 39.0%). In advanced colorectal cancers, the frequency of LOH at 1p32, 2p16, 7q31, 8p12-22, and 17q11 was significantly higher in MSI-L than that in MSS cancers. In addition, a high frequency of cancers with MSI-L phenotype was observed in early colorectal cancers (51.0%) compared with advanced lesions (25.9%) [60]. K-ras mutation was also more frequently observed in advanced MSI-L colorectal cancers, whereas no such correlation was observed in early colorectal tumors with MSI-L [60]. Others have corroborated this finding of more frequent K-ras mutations in MSI-L as compared to MSS tumors [55,59]. In each of 3 independent studies, K-ras mutation was found to be significantly more frequent in MSI-L (41% to 50%) versus MSS cancers (18% to 30%) [55,59,60]. The high frequency of K-ras mutation appears to occur in non-MSI-H cancers with the CpG island methylator phenotype (CIMP) and be associated with methylation of the DNA repair gene methylguanine transferase (MGMT) [61,63]. Whitehall et al. has reported that methylation of MGMT is associated with MSI-L colorectal cancers with instability at the D2S123

marker [44]. Loss of expression of the MGMT gene may represent the underlying molecular mechanism for MSI-L tumors [44]. Jass et al. has postulated that inactivation of MGMT may result in excessive production of methylG:T mismatches which would increase the probability of errors during DNA resynthesis that escape the mismatch repair system [56].

Based on the common features of MSI-L, K-ras mutation and serrated morphology, Jass and others have suggested that the family of serrated polyps (i.e. serrated adenomas, mixed polyps, and hyperplastic polyps) and MSI-L colorectal cancer might be related histogenetically [43,64]. Given that the adenoma-carcinoma sequence in sporadic MSI-H colorectal cancer is found only rarely and that MSI in adenomas occurs mainly at a low level (MSI-L), Jass and co-workers have postulated an alternative colorectal tumorigenesis pathway termed the “serrated pathway” [64–67]. It has been observed that MSI-L status is frequent in serrated polyps showing dysplasia, but not within traditional adenomas [64]. Due to the presence of MSI-L in some hyperplastic polyps and the lack of sporadic MSI-H adenomas, Jass has suggested that many sporadic MSI-H cancers may arise along an accelerated “serrated” pathway: hyperplastic polyp to serrated adenoma to cancer. All serrated or hyperplastic polyps, however, do not appear to be at the same risk of malignant transformation. Rather, a heterogeneity in hyperplastic polyps may exist, whereby left sided and distal hyperplastic polyps may continue to be viewed as innocuous, while proximal lesions need to be considered at increased risk for colorectal carcinogenesis [43,68].

In the model proposed by Jass, proximal serrated polyps may progress to MSI-H colorectal cancer through the inactivation of HPP1 in a background of disordered methylation [43]. The precise role of HPP1 is unknown, but it appears to be involved in the regulation of $TGF\beta$, which is known to be involved in regulating growth, differentiation and cell migration [69, 70]. Those hyperplastic polyps with the MSI-L phenotype are thereby “primed” for the subsequent loss of MLH1 expression and the high mutator pathway [43]. Methylation of the DNA mismatch repair gene MLH1 would result in the MSI-H phenotype with progression to malignancy occurring rapidly through the acquisition of DNA instability [43,68]. This, in part, could explain why a paucity of MSI-H adenomas are clinically encountered.

Recently, a high frequency of BRAF mutation has been documented in serrated polyps, including both serrated adenomas and hyperplastic polyps [71]. BRAF

is one of three RAF genes that encodes a cytoplasmic serine/threonine kinase which is regulated by binding to RAS and can subsequently lead to malignant transformation. Mutation of BRAF is very infrequent in conventional adenomas. BRAF mutation, however, is known to be associated with MSI-H colorectal cancer and more recently this association has been narrowed down exclusively to the sporadic MSI-H subset [72, 73]. Since serrated polyps and sporadic MSI-H colorectal cancer are the principal colorectal lesions that have BRAF mutation, the link between the two seems certain. The high incidence of BRAF mutations in hyperplastic polyps and serrated adenomas is consistent with the notion that the majority of colorectal cancers carrying BRAF mutations have progressed through the serrated carcinoma pathway [71]. BRAF mutation is also found in the non-MSI-H subset of colorectal cancers with extensive DNA methylation, though not to the same extent as the sporadic MSI-H subset (personal correspondence). This suggests that serrated polyps may be the precursors of CIMP colorectal cancers that may be non-MSI-H as well as MSI-H.

BRAF is immediately downstream of K-ras, which is also associated with serrated polyps and CIMP colorectal cancers [74]. BRAF and K-ras mutations, however, are mutually exclusive [72]. It seems that mutation of one or the other of these closely related oncogenes is associated with CIMP status and can perhaps synergize with a gene (or genes) that are silenced by methylation. This may provide a mechanism for the early initiation of the serrated pathway. The fact that the pathway can bifurcate does not necessarily diminish its importance, but rather may increase its significance in terms of its potential overall contribution to tumorigenesis.

Recently, Whitehall et al. examined a small series of heavily methylated non-MSI-H colorectal cancers and showed that most of these colorectal cancers were MSI-L [75]. MSI-L status, however, does not appear to be restricted to the non-MSI-H CIMP-high subset of colorectal cancers. Rather, colorectal cancers with the MSI-L phenotype are more heterogeneous than the MSI-H group. In other words, some may be classified with the sporadic MSI-H subset (e.g. showing extensive methylation, either BRAF or K-ras mutation, and an origin in serrated polyps), whereas other will not. Being a small and heterogeneous subset, it will only be possible to characterize fully MSI-L colorectal cancers by applying a multilayered and meticulous approach to a very large series of cases.

One such novel approach comes from recent data derived from the use of the cDNA microarray, which

supports the use of MSI-L as a natural sub-grouping of colorectal cancers [76]. To evaluate the impact of MSI status on global molecular phenotypes and to determine the importance of MSI relative to other molecular and clinical features, Mori et al. examined 41 colon cancers using cDNA microarray [76]. Interestingly, MSI-L cancers were clearly distinguishable from non-MSI-L tumors by principle component 10 (the "MSI-L separator"). Of note, several genes identified on the basis of high loading values in the MSI-L separator component have previously been implicated in the biology of MSI-L tumors. For example, the loading value of the DNA repair gene MGMT in component 10 was negative, consistent with decreased expression of MGMT in MSI-L tumors due to promoter hypermethylation [44, 76]. Another relevant gene was selenoprotein P, which has previously been postulated as a possible cause of disrupted DNA MMR in MSI-L tumors [77]. This data, therefore, appears to substantiate the distinction between MSS and MSI-L tumors based on molecular phenotypes. Of note, the impact of the MSI-L distinction was more subtle than that of the MSI-H on molecular phenotype, which corresponds to the known clinicopathologic similarities between MSS and MSI-L tumors [28,78].

4. Continuing questions and controversies

In spite of the reports noted above, there is still controversy over whether MSI-L tumors exist as a distinct sub-group of colorectal cancer. Some authors have argued that because microsatellite repeat slippage occurs about every 1,000 to 10,000 divisions in normal cells, the clonal nature of cancers would make this slippage detectable and therefore account for at least some MSI-L tumors [58]. That is, given the use of enough makers, it may be that all colorectal cancers will exhibit some level of MSI [37]. In two independent studies examining sporadic colorectal carcinomas, 68% and 79% of the non-MSI-H tumors exhibited some MSI and therefore could nominally be classified as MSI-L [57,58]. In the study by Halford et al., however, no evidence for a discrete MSI-L group was identified. No association was found between MSI-L (or the level of MSI) and any clinicopathological or molecular variable, including K-ras mutation and LOH at APC [57]. A recent meta-analysis of those studies investigating the relation of K-ras mutation and MSI-L similarly failed to show a significantly higher frequency of K-ras in MSI-L cancers. Rather a significant heterogeneity was

found in the frequency of K-ras mutations in MSI-L and MSS tumors, presumably some of which was due to the different methods used to assess MSI in each group [38]. Critics of the MSI-L sub-classification suggest that the greater variation in the non-MSI-H group may be more due to MSI-L cancers being quantitatively different from MSS tumors in some way (i.e. larger number of cell divisions, somatic mutational load, or germ-line genotype), rather than there actually being a true qualitative distinction from MSS cancers [38,58]. In this regard, Gonzalez-Garcia et al. specifically considered whether the frequency of MSI in the cancers they examined better fit a bimodal (MSI positive, MSS) or trimodal (MSI-H, MSI-L, and MSS) distribution and found no evidence for a separate category for MSI-L cancers [38,79].

Finally, some authors have questioned the validity of the “serrated adenoma pathway” and the role of MSI-L in sporadic colorectal carcinogenesis. Although a “serrated pathway” of colorectal carcinogenesis may exist, critics of MSI-L’s role in tumorigenesis suggest that an alternative explanation for the reason that MSI-L is found very rarely in sporadic, non-serrated adenomas is that MSI-H do develop from classical adenomas, but that MSI is not acquired until after malignant progression [38]. In support of this, Sawyer et al. analyzed 39 serrated adenomas from 27 patients to define the molecular characteristics of serrated adenomas of the colorectum [80]. MSI was found in < 5% of cases and there was no loss of expression of MMR proteins. Instead, a variety of genetic defects including APC mutation/LOH, β -catenin mutation and CRAC1 LOH were found in a number of serrated adenomas. Given these findings, the authors concluded that no “serrated pathway” per se existed, but rather the pathway of colorectal tumorigenesis for serrated adenomas appeared to be heterogeneous and could not be accounted for by changes in known genes [80].

5. MSI status and prognostic implications

It is clear that MSI status has prognostic implications. Retrospective and population-based studies have been published confirming an improved survival in patients with MSI-H versus non-MSI-H colorectal tumors. In the Ontario population-based study, a series of 607 patients were diagnosed with colorectal cancer at age 50 or younger. Multivariate analysis revealed that MSI-H was an independent prognostic factor, along with stage at diagnosis, histology of tumor, and tumor grade [46].

Furthermore, regardless of the depth of tumor invasion, colorectal cancers with high-frequency MSI had a decreased likelihood of metastasizing to regional lymph nodes or distant organs [46]. Based on the findings of this study, high-frequency MSI in colorectal cancer appears to be independently predictive of a relatively favorable outcome [46]. Similarly, in the Utah and Northern California population-based study of 1,026 patients, there was a relationship between MSI-H and improved prognosis independent of stage, site, tumor grade, and age [81]. In fact, the authors reported that there was a 60% decrease in colorectal-related deaths in patients with MSI-H tumors. Most of the risk reduction occurred in Stage III patients whose tumors behaved as Stage II if the tumor was MSI-H [81]. A retrospective analysis of 460 patients with Stage III colorectal cancer, enrolled in five national trials of adjuvant 5-fluorouracil (FU)-based chemotherapy, revealed that patients with MSI tumors and mutations in the type II receptor for TGF- β 1 had a better prognosis [82]. It therefore appears that not all MSI-H tumors behave the same. In one study, mutation of the gene for the type II receptor for TGF- β 1 in cancers with high levels of microsatellite instability had a more favorable outcome [81,82].

Even though the majority of the published data suggest that colorectal cancer patients with MSI-H tumors have an improved prognosis, there are still some conflicting reports. Investigators from Scotland recently reported that patients less than 30 years of age at diagnosis who had MSI-H colorectal cancer had a worse chance of survival than those patients whose tumors were MSS [83]. Younger patients had excess MSI tumors, mucinous tumors, and more advanced disease [83]. The relative risk of death associated with tumor MSI was 0.87 in the cohort of patients < 30 years of age as compared to 0.11 for the control cohort of older patients [83].

MSI status also appears to play a critical role in the responsiveness of colorectal tumors to certain types of chemotherapy [84]. Recently a study by Ribic et al. showed that fluorouracil-based adjuvant chemotherapy benefited patients with stage II or stage III colon cancer with MSS or MSI-L, but not those with tumors exhibiting MSI-H [84]. In the absence of adjuvant chemotherapy, the patients with tumors displaying high-frequency microsatellite instability had significantly longer overall survival than patients with tumors exhibiting microsatellite stability or low-frequency microsatellite instability. When the analysis was limited to the group receiving adjuvant chemotherapy, however, patients with MSI-H tumors did not have a significant increase in

overall survival as compared with patients with MSS or MSI-L tumors [84]. Therefore, it appeared that, opposed to the patients with non-MSI-H tumors, the MSI-H group did not benefit from the adjuvant chemotherapy.

Although there is a preponderance of data indicating a significant difference in the prognosis of MSI-H versus non-MSI-H tumors, currently there is no data to support a distinction between MSI-L and MSS cancers with regards to prognosis. Rather, tumors with low-frequency microsatellite instability have been found to have similar clinical and prognostic features to MSS tumors [46,53,79,84]. In multiple studies, a bimodal distribution of MSI (MSI-H versus non-MSI-H), rather than a trimodal distribution (MSI-H versus MSI-L versus MSS) has been shown to more accurately reflect the prognosis and natural history of colorectal tumors [46, 79,84].

6. Conclusion

MSI is a well-recognized and accepted phenomenon. The existence of MSI-H tumors as a unique subclassification of colorectal tumors has been unequivocally established both phenotypically and genetically. What remains to be determined is whether an intermediate classification of MSI-L tumors exists between those tumors classified as MSS and MSI-H. To date, the MSI-L subset has not shown any distinct clinical or morphological differences from the remaining MSS cancers. Given this, some may wonder why any debate around the existence of MSI-L status is even clinically relevant or warranted. MSI-L status is important insofar as MSI-L might serve as the by-product of a DNA repair defect. Identifying this defect, however subtle it may be, could hold potential importance as it may define a subgroup of tumors associated with a distinct natural history and an altered response to chemotherapeutic agents. Because of this, the possibility that a "mild mutator" pathway may be driven by a DNA repair defect in MSI-L tumors warrants additional scientific and clinical research. It is imperative that future prospective studies not only evaluate the role of MSI as a prognostic factor, but that these studies also examine specific mutations in an attempt to sub-classify MSI-L with regard to MSS tumors so that subtle differences in prognosis and natural history between these two sub-groups can be identified.

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